Master of Public Health

Master de Santé Publique

Evaluating the Impact of Cold Chain Deviations on the Risk of Hepatitis B Infection in Children: A Study Utilizing Fridge-Tag2® Temperature Monitors for the Hepatitis B Birth Dose Vaccine in Burkina Faso

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EUROPUBHEALTH+
2021-2024
Epidemiology and Biostatistics

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Acknowledgments

Firstly, I would like to express my heartfelt gratitude to my family for their unwavering support and encouragement.

I also want to thank my professional advisor, Dr. Yusuke Shimakawa, and my academic advisor, Dr. Rebecca Kehm, for their invaluable support and guidance. Their insights and encouragement have been instrumental throughout this journey.

I would also like to extend my thanks to the NéoVac team, particularly Abdoul Tiendrebeogo, Yanis Dahoumane, and Laura Schaeffer for their invaluable assistance in providing the data and overall support. Additionally, I am profoundly grateful to everyone at Institut Pasteur, with special thanks to Lucia for being exceptionally helpful.

A thesis submitted in conformity with the requirements for the degree of

MASTER OF PUBLIC HEALTH

At Ecole des Hautes Etudes en Santé Publique, France.

June 2024

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List of Acronyms

Anti-HBs:	Antibody of HBsAg
CRF:	Case Report Form
CDI:	Cumulative Deviation Index
CTC:	Controlled Temperature Chain
CSPS:	Centre de Santé et de Promotion Sociale
GSK:	GlaxoSmithKline
HBsAg:	Hepatitis B Surface Antigen
HBV:	Hepatitis B virus
HBeAg:	Hepatitis B e antigen
HBsAg:	Hepatitis B surface antigen
HepB vaccine:	Hepatitis B vaccine
HepB3:	Three doses of pentavalent, DTP-HepB-Hib series
ICC:	Inside-Cold-Chain
IPAC:	The WHO Immunization Practices Advisory Committee
MenAfriVac:	A vaccine developed for use in Africa against meningococcal bacterium Neisseria meningitidis group A.
NéoVac:	Néonatal Vaccination against Hepatitis B in Africa
OCC:	Out-of-cold chain
RDT:	Rapid Diagnostic Test
TADES:	Anti-HBs titration in children with incomplete vaccination

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Abstract – English

Background: Maintaining the recommended cold chain of 2-8°C for vaccine storage and transport is challenging, especially in resource-poor settings. Timely birth dose administration is crucial to prevent mother-to-child transmission of hepatitis B virus (HBV). The monovalent hepatitis B vaccine has demonstrated heat stability in in-vivo and in-vitro studies. However, the impact of out-of-cold chain storage on vaccine effectiveness and immunogenicity has not been evaluated in real-world decentralized settings in Africa.

Objective: This study assessed real-world temperature exposures of the monovalent hepatitis B birth dose and the impact of temperature deviations on vaccine effectiveness and immunogenicity in Burkina Faso.

Method: This prospective cohort study was nested within the NéoVac (Néonatal Vaccination against Hepatitis B in Africa) stepped-wedge cluster randomized trial in Burkina Faso, evaluating the impact of introducing HepB-BD on the risk of child HBV infection. Continuous temperature monitoring data were collected across 23 primary healthcare facilities using Fridge-tag$^{2®}$ devices. Serological outcomes, including hepatitis B surface antigen (HBsAg) status and anti-HBs, were analyzed from the NéoVac and TADES (Anti-HBs titration in children with incomplete vaccination) studies in relation to vaccine temperature exposures. The primary outcome was the proportion of HBsAg-positive children after 9 months.

Results: Deviations from the recommended cold chain conditions (2-8°C) were observed, with 30.5% of days exceeding 8°C and 1.9% below 2°C. Maternal hepatitis B viral load was the main risk factor for child HBsAg positivity. After adjustment for risk factor, no association between vaccine temperature exposure and child HBsAg status was found. Children receiving the HepB-BD vaccine had higher rates of seroprotection, anti-HBs $\geq$10mIU/mL, (90.0% vs. 77.8%, OR: 2.3, 95% CI: 0.4-14.8, p-value: 0.326) and anti-HBs $\geq$100mIU/mL, (91.0% vs 33.3%, OR: 7.1, 95% CI: 1.8-31.8, p-value:0.004) compared to those who did not receive the birth dose.

Conclusion: The monovalent hepatitis B birth dose vaccine retained immunogenicity despite high-temperature exposure, likely due to its inherent thermostability. However, no vaccine in the TADES study was exposed to low temperatures, and the impact of freezing temperatures on vaccines was inconclusive.

Keywords: hepatitis B vaccine; mother-to-child transmission; birth dose vaccination; temperature deviation

RESUME

Contexte: Le maintien de la chaîne du froid recommandée de 2 à 8°C pour le stockage et le transport des vaccins est un défi, en particulier dans les régions à faibles ressources. L'administration en temps voulu de la dose à la naissance est cruciale pour prévenir la transmission du virus de l'hépatite B (VHB) de la mère à l'enfant. Le vaccin monovalent contre l'hépatite B a démontré sa stabilité à la chaleur dans des études in-vivo et in-vitro. Cependant, l'impact du stockage en dehors de la chaîne du froid sur l'efficacité et l'immunogénicité des vaccins n'a pas été évalué dans des contextes réels décentralisés en Afrique. **Objectif :** Cette étude a évalué l'exposition à la température du vaccin monovalent contre l'hépatite B administré à la naissance et l'impact des écarts de température sur l'efficacité et l'immunogénicité du vaccin au Burkina Faso. **Méthodes:** Cette étude de cohorte prospective s'inscrit dans le cadre de l'essai randomisé en grappes à plusieurs niveaux NéoVac (Vaccination néonatale contre l'hépatite B en Afrique) au Burkina Faso, qui évalue l'impact de l'introduction de l'HepB-BD sur le risque d'infection par le VHB chez l'enfant. Des données de surveillance continue de la température ont été recueillies dans 23 établissements de soins de santé primaires à l'aide d'appareils Fridge-tag2®. Les résultats sérologiques, y compris le statut de l'antigène de surface de l'hépatite B (HBsAg) et de l'anti-HBs, ont été analysés à partir des études NéoVac et TADES (titrage de l'anti-HBs chez les enfants dont la vaccination est incomplète) en relation avec l'exposition à la température du vaccin. Le résultat principal était la proportion d'enfants AgHBs positifs après 9 mois. **Résultats:** Des écarts par rapport aux conditions recommandées de la chaîne du froid (2-8°C) ont été observés, avec 30,5 % des jours dépassant 8°C et 1,9 % en dessous de 2°C. La charge virale de l'hépatite B chez la mère était le principal facteur de risque de positivité de l'Ag HBs chez l'enfant. Après ajustement des facteurs de risque, aucune association n'a été trouvée entre l'exposition à la température du vaccin et le statut de l'enfant vis-à-vis de l'Ag HBs. Les enfants ayant reçu le vaccin HepB-BD présentaient des taux plus élevés de séroprotection, d'anti-HBs >10mUI/mL, (90,0% vs. 77,8%, OR : 2,3, 95% CI : 0,4-14,8, p-value : 0,326) et d'anti-HBs >100mUI/mL, (91,0% vs 33,3%, OR : 7,1, 95% CI : 1,8-31,8, p-value:0,004) par rapport à ceux qui n'avaient pas reçu la dose à la naissance. **Conclusion:** Le vaccin monovalent contre l'hépatite B administré à la naissance a conservé son immunogénicité malgré des expositions à des températures élevées, probablement en raison de sa thermostabilité inhérente. Cependant, aucun vaccin de l'étude TADES n'a été exposé à de basses températures, et l'impact de la température de congélation sur les vaccins n'a pas été concluant.

Mots clés : vaccin contre l'hépatite B ; transmission mère-enfant ; vaccination à la naissance ; écart de température.

Translated with DeepL : (1)

Introduction

Background

Hepatitis B virus (HBV) is a serious public health problem. In 2022, the World Health Organization (WHO) estimated that 254 million people worldwide were living with a chronic HBV infection. This has resulted in 1.1 million deaths, primarily from cirrhosis and hepatocellular carcinoma (2). The prevalence of chronic HBV infection was the highest in the African (6.1%) and Western Pacific Regions (6.2%) in 2015 (3).

Chronic HBV infection is caused by the hepatitis B virus and is present in a wide array of bodily fluids including blood, saliva, semen, vaginal secretions, menstrual blood, sweat, breast milk, tears, and urine of infected individuals (4). This allows the virus to transmit through multiple routes. These routes include mother-to-child transmission (MTCT) and horizontal transmission from mucosal contact with infectious body fluids. Mother-to-child transmission is one of the main modes of transmission in regions with high HBV endemicity (5).

The risk of chronic HBV infection, following exposure to the virus, is inversely correlated with age at infection. Children infected at birth through MTCT have an 80%-90% risk of developing chronic HBV infections, compared to only 1%-5% of adults who become infected (6). Furthermore, those who develop chronic HBV infection through MTCT have a higher risk of severe clinical outcomes compared to those infected through horizontal transmission (7).

The prevention of MTCT of HBV is important in eliminating hepatitis B as a public health threat (8). The risk of vertical transmission of the virus is 70% to 90% for children who have mothers with a high HBV viral load or who tested positive for the hepatitis B e antigen (HBeAg) in the absence of any preventive measure (8, 9). The WHO recommends that all children receive the hepatitis B vaccine as soon as they are born, preferably within 24 hours (8). According to a meta-analysis of randomized controlled trials, children of HBV-positive mothers who received the first dose of the vaccine at birth had a 3.5-fold lower risk (RR: 0.28, 95% CI: 0.2-0.4) of infection than children who did not receive any dose (9).

Hepatitis B Vaccines

The hepatitis B vaccine (HepB vaccine) is a significant public health achievement and has been included in the national vaccination program of nearly all countries worldwide, 97% (6). The earliest HepB vaccine was developed by Krugman et al. in the late 1960s (10). Hepatitis B virus contains 3 important antigens: c, e and s (8). The active component of all HepB

vaccines is the hepatitis B surface antigen (HBsAg) protein. Antibody of HBsAg (anti-HBs) is used as a marker for immunity against HBV (11).

The protective efficacy of the hepatitis B vaccine is primarily measured by an anti-HBs titer of greater than 10 mIU/mL, measured 1-2 months after the administration of the last dose of the primary vaccination series (8). This concentration is considered a reliable serological marker of long-term protection against HBV infection. On the other hand, seroprotection is a measure of the mean concentrations of anti-HBs and the proportions of vaccines that are seroprotected (11, 12).

Countries that added the vaccine to their vaccination program have seen a reduction in both the prevalence of HBsAg and the incidence of related complications such as hepatocellular carcinoma (6). In 2019, the prevalence of HBsAg in children under five years old reduced from 4.7% before vaccines were introduced to under 1% (6) in Cameroon. Other factors such as the increase in maternal age of childbirth and a decrease in the number of siblings could also contribute to the reduction of chronic HBV infection prevalence (13). However, this reduction can be attributed to the widespread use of the hepatitis B vaccine as the greatest reduction is in the Western Pacific region, from 8.3% HBsAg prevalence in the pre-vaccine era to 0.93% in 2002-2015 (6). Moreover, mathematical models estimated that HBV vaccination programs have averted 210 million new HBV infections globally (6).

In 2021, the WHO African region, with one of the highest prevalence of HBV infection had the least coverage for HepB-BD vaccination; only 13 of the 48 countries had established routine HepB-BD vaccination policy as of April 2021 (14). All WHO African countries introduced the 3 doses of HepB (HepB3) in 2014, but the number of countries with ≥90% HepB3 coverage declined from 43% in 2018 to 34% in 2021 (15). Increasing this coverage is important in the aim of eliminating mother-child transmission of HBV.

Challenges in Delivering Hepatitis B Birth Dose

The provision of a potent first dose of the HepB vaccine within 24 hours of delivery presents a unique challenge, particularly in countries where significant percentages of births are at home. National immunization programs may be interested in facilitating outreach vaccine delivery through traditional birth attendants or other community health workers for children born outside healthcare facilities. But the implementation of this strategy may be hindered by the lack of cold storage facilities (14, 16). The introduction of an out-of-the-cold-chain (OCC) strategy for heat-stable vaccines has the potential to extend the reach and increase coverage by allowing outreach HepB-BD administration (16, 17).

Thermostability of the Hepatitis B Vaccines

The WHO guidelines and the HepB vaccine manufacturers recommend storage of 2-8°C for the vaccine (8, 18). However, studies have shown that HepB vaccine is heat stable and can be stored OCC for certain periods without significant loss of potency (16). The WHO Immunization Practices Advisory Committee (IPAC) strongly recommends that countries only use vaccines at temperatures above the standard cold chain range (2-8°C) if those vaccines have been specifically licenced and labeled for use in a Controlled Temperature Chain (CTC) (19). The Hepatitis B vaccine was the initial vaccine chosen for licensing and labeling by CTC protocols, recognizing its potential to facilitate timely administration of the birth dose and its efficacy in out-of-cold chain settings (19). The process of standardizing stability testing and regulatory approval for CTC use of the Hepatitis B vaccine was complex due to differences in product formulations among manufacturers (19). Consequently, the focus shifted to the MenAfriVac vaccine for Meningitis A which successfully became the first vaccine to be licensed for CTC use in 2012, setting a precedent for other vaccines (19, 20). MenAfriVac is being widely deployed across the meningitis belt with significant success, frequently attaining high levels of vaccination coverage and effectively preventing outbreaks of meningitis type A (20). The CTC approach has allowed vaccinators to conduct more outreach and mobile vaccination in hard-to-reach areas without being constrained by the need to maintain the cold chain (2-8°C) all the time (20).

The successful implementation of the MenAfriVac vaccine under the CTC model sets a compelling precedent for the inclusion of other vaccines, such as Hepatitis B, in the CTC framework. Given that Hepatitis B is identified as a priority in the CTC roadmap, the achievements with MenAfriVac should significantly bolster efforts to license and deploy HepB-BD under similar conditions (17, 20). The demand for CTC-licensed hepatitis B vaccines among countries in African and Western Pacific regions was highlighted in a recent survey, where 72% indicated that CTC would facilitate the provision of HepB-BD. Countries with a high percentage of home births appeared to see CTC as a potential facilitator of the provision of HepB-BD (21).

A WHO systematic review of monovalent hepatitis B vaccine thermostability, as well as various field and laboratory studies conducted provide a comprehensive understanding of the vaccine's heat stability and potential benefits of OCC. The WHO systematic review included data from eight manufacturers and showed that monovalent hepatitis B vaccines retained in vitro potency and met other stability criteria after exposure to temperatures of 37°C for 4-6

weeks and to 45°C for shorter periods. Experimental healthy adult studies, including those by Just et al. and Van Damme et al., have shown that heating the vaccine for one week at 45°C or for one month at 37°C did not alter its reactogenicity or its ability to elicit protective antibody titers. These findings are significant for situations where the cold chain might be compromised (16).

Field studies have demonstrated the practicality of OCC storage for the HepB vaccine (12, 22-24). A study conducted by Hipgrave et al in rural Vietnam compared the immunogenicity of a locally produced vaccine among children who received three doses stored within the cold chain (n=358) or for whom the first dose was stored OCC for up to one month (n=748) (12). The results demonstrated that there were no significant differences in the prevalence of a protective level of antibodies (≥ 10 mIU/mL) or antibody titer among groups of children. This finding is consistent with other studies that have reported similar immunogenicity between Hep B vaccines stored OCC compared to those maintained within recommended temperature ranges (23, 24).

A study conducted in rural townships across three counties in Hunan Province, China, randomized three groups with different strategies for delivery of the first dose of the HepB vaccine. The townships were divided into three groups: Group 1 vaccines were stored within the cold chain and administered in the township hospital; Group 2 vaccines were stored out of the cold chain in villages and had it administered by village-based health workers at home; Group 3 followed the same protocol as Group 2 but a prefilled inject device was used for administration (23). No significant differences in antibody response to vaccine were observed between the groups. In 2016, Kolwaite et al conducted non-randomized trials in Lao PDR, where the monovalent hepatitis B vaccine was stored at room temperature for up to 28 days. The study enrolled two cohorts of children; 388 children aged 2-8 months and 317 children aged 14-20 months and compared HepB-BD coverage (24). The results showed a median 27% increase in the Hep-BD (IQR 58%, $p < 0.0001$) in the OCC setting (24)

The thermostability of the HepB vaccine is important in its storage and distribution and can be leveraged in resource-poor settings where maintaining a consistent cold chain can be challenging (10, 12). Access to a potent birth dose of the vaccine, which is essential for preventing mother-to-child transmission of HBV and subsequent chronic infection, in remote areas lacking refrigeration facilities can be increased by the heat stability of the vaccine (10).

Problem Statement

The effective prevention of hepatitis B virus transmission from mother-to-child is a crucial public health goal. Burkina Faso, a West African country is considered highly endemic for active HBV as the prevalence of HBsAg in the general populations is 10%, which is above the threshold prevalence of ≥8% that defines a highly endemic area (8, 25). While the administration of the HepB-BD vaccine at birth is a key intervention in this effort, the maintenance of the cold chain poses significant logistical challenges that can hinder timely vaccine delivery, especially in remote and resource-limited settings where many births occur at home (14).

Research Gap

While there is evidence to support the thermostability of the HepB vaccines, a critical gap remains in our understanding of the real-world temperature exposure experienced by the monovalent HepB vaccines and their impact on vaccine effectiveness and immunogenicity. Existing field studies have not comprehensively examined the actual temperature conditions experienced by the monovalent HepB vaccines. Therefore, this study presents a unique opportunity to address this gap by utilizing Fridge-tag $^{2®}$ (Fridge-tag) monitored data to assess the immunogenicity of HepB vaccines exposed to temperatures outside the recommended cold chain conditions. Fridge-tag is an innovative temperature monitoring technology that can be used to gather details on the temperatures to which the vaccine was exposed and the average duration of OCC storage before use (18). This approach allows for understanding the association between temperature exposures and key HBV infection outcomes such as HBsAg proportion and antibody levels in children who received the birth dose.

Moreover, existing evidence is largely derived from laboratory experiments, studies in healthy adult populations, or field studies outside of the African context. In contrast, this study will provide valuable real-world insights specific to the African region, where challenges in maintaining the cold chain is present. Bridging this contextual evidence gap is crucial for informing immunization policies and practices that are tailored to the resource-constrained settings prevalent across Africa.

Specific Condition to which work was conducted and contributions made.

This work was conducted at Institut Pasteur under the supervision of Dr. Yusuke Shimawaka. Data entry, compilation, merging, analyses, and writing were all carried out by Fadumo Abdullahi with guidance from Dr. Shimawaka. Dr. Shimawaka, MD, PhD, DTM, MSc, is a senior epidemiologist at Institut Pasteur and an expert on viral hepatitis control in Africa. He is the project coordinator for the NéoVac and TADES studies.

Main Objectives

General Objective: To evaluate the impact of real-world temperature exposures on the effectiveness (proportion of children positive for HBsAg) and immunogenicity (anti-HBs) of the monovalent hepatitis B birth dose vaccine.

Specific Objectives:

- To describe the temperature vaccines were exposed to across the various Centre de Santé et de Promotion Sociale (CSPS)
- To assess the effectiveness of the monovalent hepatitis B birth dose vaccine on the risk of MTCT, defined as positive HBsAg in children aged 9 months.
- To evaluate the immunogenicity of the hepatitis B vaccine in children who received HepB-BD dose of vaccine exposed to temperatures outside the recommended cold chain parameters, by comparing the levels of hepatitis B surface antibodies (anti-HBs) after vaccination. An anti-HBs level of 10 mIU/mL or higher was considered protective.
- To examine the impact of introducing the HepB-BD exposure to cold chain temperature deviations on the risk of MTCT of HBV among chidlren born to HBsAg-positive mothers who received the birth dose vaccine under varying temperature storage conditions.

Materials and Methods

Study Design

This study was nested within a larger framework of the Néonatal Vaccination against Hepatitis B in Africa (NéoVac) study, a stepped wedge cluster randomized controlled trial conducted in the Hauts-Bassins Region of Burkina Faso (26). The NéoVac study aimed to evaluate the impact of introducing the hepatitis B birth dose vaccine in Burkina Faso, where the hepatitis B vaccination is currently scheduled at 8-12-16 weeks. This study utilized a subset of the NéoVac data, focusing on the effectiveness of introducing the monovalent HepB-BD combined with the three doses of the child hepatitis B vaccine where the HepB-BD real-world temperature exposures were known.

Additionally, the study used another dataset from a multicentre, cross-sectional study, Anti-HBs titration in children with incomplete vaccination in Burkina Faso (TADES). The TADES study aimed to assess the level of HBV immunization (anti-HBs titer) in children aged 12 to 36

months with an incomplete vaccination schedule as one or two doses of HBV vaccine. The sample control group of this study consisted of children of the same age group attending the same Centre de Santé et de Promotion Sociale (CSPS) and some of these children were participants of the NéoVac study. The CSPS are primary healthcare facilities that pregnant women visit for antenatal care, child delivery and child immunization.

The NéoVac study was designed to introduce the HepB-BD vaccine in 24 Centre de Santé et de Promotion Sociale (CSPS) located in the Hauts-Bassins Region of Burkina Faso. At regular intervals, one CSPS was randomized to add HepB-BD to the immunization schedule, and the process was repeated until all 24 CSPS added HepB-BD administration into the schedule.

This study was a prospective cohort study focusing on a cohort of children who received the monovalent HepB-BD vaccine from January 2021 to August 2022. The study population was children born during the NéoVac intervention period, who have received at least the HepB-BD vaccine.

Study Setting

The NéoVac study was conducted in two districts in the Hauts-Bassins Region, called Dô and Dafra, which had respective populations of 548,000 and 348,000 in 2017. Twenty-four Centre de Santé et de Promotion Sociale (CSPS) were selected based on eligibility that they were in the rural/semi-rural zone of the districts and were accessible to the NéoVac study research center (Centre Muraz, located in Bobo-Dioulasso).

Study Participants

NéoVac study participants: In the NéoVac study, a total of 3,715 children (92.9% of the 4,044 mother/child pairs born during the intervention period) were vaccinated during the intervention period at various CSPS. However, the analysis presented in this thesis is on a subset of 2,110 children due to several exclusions.

The exclusions were as follows (Figure 1):

- 23 children died before 9 months of age
- 4 children died after 9 months of age
- 1,120 children were excluded due to lack of vaccine inventory data
- 458 children were further excluded due to missing HBsAg data

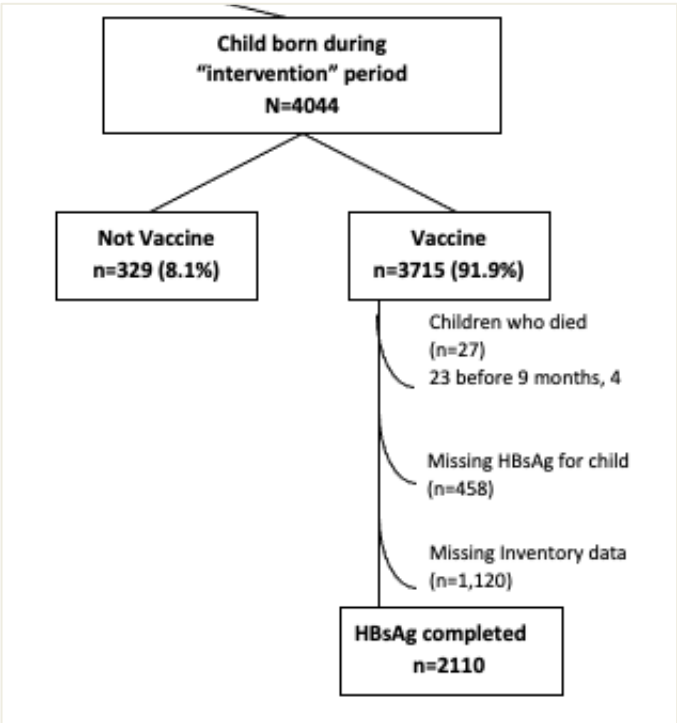

Figure 1: Flow Chart of the NéoVac Study Participants

TADES study participants (Figure 2)**:** In the TADES study enrolled a total of 147 children who had anti-HBs levels measured. From the unvaccinated case group (n=115), 42 children of them were participants of the NéoVac study and were included in the analysis of this study. Among the vaccinated control group (n=32), 26 children of them were participants NéoVac study and had vaccine temperature data.

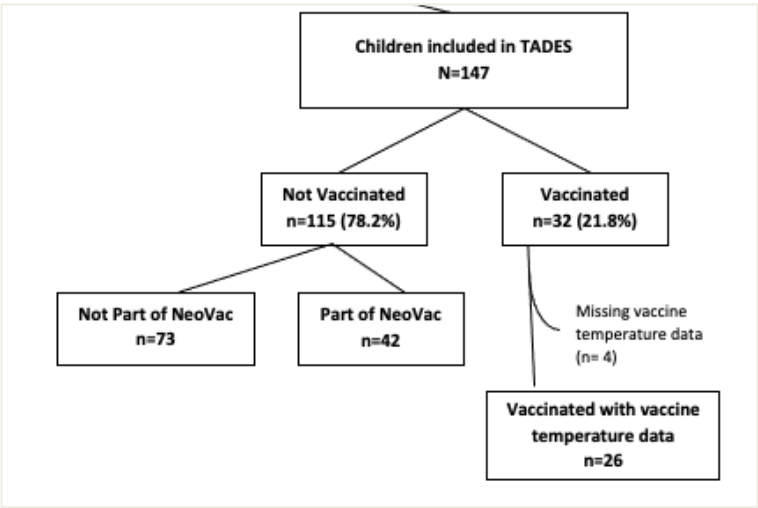

Figure 2: Flow Chart for the TADES Study Participants

Vaccine

The hepatitis B recombinant vaccine used in the study was an injectable suspension for intermuscular use, Engerix-B 10 µg/0.5 mL.

Cold Chain Management

The monovalent vaccines were purchased by the research team and were transported by supplier GSK from France to Burkina Faso by air. This stage's quality controls were conducted in compliance with the supplier's requirements for cold chain management and transportation of the vaccine's cold chain and temperature was continuously monitored. The vaccine was transported to Muraz Center by refrigerated tracks. After the arrival of vaccines at the Muraz Centre, they were kept in refrigerators with continuous temperature records and Fridge-tag alarms, and a monthly summary of the temperature curves was produced. The districts were supplied once every two months. Then the districts supplied the CSPS. In some cases, the Muraz Center supplied CSPS directly.

The WHO guidelines for vaccine cold chain management recommends destroying the hepatitis B vaccines if they are exposed temperatures above 8°C for at least 14 days, and if exposed to temperatures below -0.5°C for at least 1 hour, an agitation test to be conducted to determine if the vaccine should be stored or destroyed. The rules for temperatures above 8°C were more lenient as the WHO recommends the destruction of the hepatitis B vaccines if they are exposed to temperatures above 8°C for at least 14 days (27). As per the protocol, in the event of a deviation above 8°C, the vaccines were destroyed following the WHO guideline. During the agitation test, a deviation from the protocol occurred. The Engerix-B 10 µg/0.5 mL, which was the original vaccine specified for the test, was not utilized to conduct the agitation test due to its limited volume of 0.5 mL per dose. As an alternative, the DTP-HepB-Hib was used as a proxy to conduct the agitation test because the vial contained 5 mL.

Temperature Monitoring with Fridge-tag Devices

Temperature monitoring of the HepB-BD cold chain was conducted using Fridge-tag electronic temperature monitoring devices. Fridge-tag took a daily temperature reading every minute. It recorded the daily minimum and maximum temperatures reached and the exact date, time, and duration of the first temperature threshold violations if an alarm limit was exceeded. If an alarm was triggered, for any temperature outside 2-8°C, the device continued to accumulate each additional temperature excursion. The temperature data for the last 60 days were downloaded as a PDF report.

The Fridge-tag devices were programmed with the recommended temperature range of 2-8°C for hepatitis B vaccine storage. The device provided detailed, continuous monitoring of the vaccine storage temperatures at the CSPS. The device's ability to record minimum, maximum,

and alarm temperatures, as well as the duration of excursions, has enabled through assessment of cold chain performance and identification of potential issues.

Data Sources

1. Clinical Data from the Stepped Wedge Cluster Randomized Trial of the NéoVac Study: This dataset contained all clinical data, including specific vaccine lot administered to each child. This dataset served as the foundation for linking vaccine exposure to immunogenicity outcomes

2. Anti-HBs titration in children with incomplete vaccination in Burkina Faso (TADES): This dataset contained children who were vaccinated with the monovalent HepB-BD as part of NéoVac and their corresponding anti-HBs titer as they were the control group for the TADES study.

3. Temperature Monitoring Data: Utilizing Fridge-tag monitors with alarms, this dataset provided a continuous record of the temperature conditions of the vaccines at each vaccination CSPS. This data was critical for identifying deviations from the recommended cold chain temperature ranges.

4. Inventory Database: This dataset included information on the arrival dates and lot numbers of vaccines at each CSPS. It was essential for establishing the timeline of vaccine availability at each CSPS and was crucial in linking the datasets.

Data Compilation

A unique database was created to trace individual vaccines, using the inventory database by assigning each vaccine an identification number. Vaccines were then linked to children based on the lot number and the date the vaccine was present at the CSPS, assuming a first-in, first-out approach for vaccine administration. The temperature-monitored data and the database created were merged using the CSPS name and data was filtered out for the vaccine to include only those days each vaccine was in the fridge.

Data entry and compilation were carried out using Excel and subsequent steps of data merging, management, cleaning, and analysis were done using R programming (version 4.3.1) (R Core Team, 2023).

Exposure Ascertainment

The exposure ascertainment in this study involved two main approaches:

1. Temperature Range Deviation:
    - Stringent exposure criteria were used to classify temperature deviations outside the recommended 2-8°C range:
        - Always normal: Always within the 2-8°C range
        - Only cold: All temperatures were <8°C, with at least one event < 2°C
        - Only hot: All temperatures were > 2°C , with at least one event >8°C
        - Mixed cold and hot: At least one event >8°C and another event < 2°C
    - A flexible exposure criteria was used to categorize temperatures:
        - Always within 2-8°C range (Unexposed)
        - Low-risk temperature exposure:
            - Between $\geq$ -0.5°C and +2°C
            - Between 8°C and $\leq$ 30°C
        - High-risk temperature exposure:
            - Freezing risk < -0.5°C
            - Heat risk > 30°C
2. Cumulative Deviation Index (CDI) Calculation:
    - The CDI is a novel metric designed to integrate both the duration and severity of temperature deviations recorded by Fridge-tag monitor devices.
    - Temperature exposure categories were defined based on evidence of vaccine stability. In vitro and vivo studies showed that vaccines exposed to “mild” freezing (-4°C or warmer) temperatures did not freeze (28). However, the WHO considers the HepB vaccine freezing point to be at -0.5°C (18).
        - Within recommended range (2-8°C): weighting factor= 0
        - Low heat exposure (>8 to <20°C): weighting factor= 1
        - Moderate heat exposure ($\geq$20-37°C): weighting factor =2
        - High heat exposure ($\geq$37°C): weighting factor= 3
        - Moderate freezing exposure (<2 to $\leq$-0.5°C): weighting factor= 4
        - High freezing exposure (< -0.5°C): weighting factor= 6
    - Integrating Weighting Factors and Exposure Duration: The CDI for each vaccine dose was calculated by multiplying the duration of exposure (hours) in each category by the corresponding weighting factors and summing these values to get a combined CDI for each vaccine.

Outcome Ascertainment

1. Effectiveness of vaccine: The effectiveness of the HepB-BD, particularly when exposed to temperatures outside the recommended cold chain conditions, was evaluated by the

proportion of children post-vaccination positive for HBsAg. This outcome was dichotomized to HBsAg positive and HBsAg negative children.

2. Immunogenicity (level of Anti-HBs): The immunogenicity of the HepB-BD, particularly when exposed to temperatures outside the recommended cold chain conditions, was evaluated through the measurement of hepatitis B surface antibodies (anti-HBs) in children post-vaccination. As part of the TADES study, blood samples were collected from vaccinated children aged 9 months and anti-HBs levels were quantified. An anti-HBs level of 10mlU/mL or higher was considered a protective immune response.

Statistical Analyses

The temperature exposure of the HepB-BD at the CSPS level was assessed through descriptive analyses. This initial analysis was presented for the temperature experienced by the refrigerators in each of the CSPS, including the number of days the temperatures fell within the defined temperature categories. As part of the descriptive analysis, characteristics of the children in the study were presented. Secondly, the proportion of children testing positive for HBsAg was calculated for the stringent and flexible temperature categories described above. A logistic regression model was used to assess the immunogenicity of the HepB-BD and temperature deviations. The model adjusted for potential confounders such maternal HBV viral DNA levels, and maternal HBsAg status. The results were presented as adjusted odds ratios with 95% confidence intervals. In cases where certain temperature categories did not have any vaccines fall into them, Firth's penalized likelihood model was used. This model addresses issues that arise from small sample size or spare data, providing more reliable estimates when dealing with rare events or complete separation of data points. The CDI described above was calculated for each vaccine dose and descriptive analyses was carried out.

The serological outcomes from the TADES study were assessed by measuring the anti-HBs titers, a marker of vaccine-induced immunity. Seroprotection was defined as an anti-HBs levels of $\geq$10 mlU/mL.

Ethical Consideration

The NéoVac and TADES studies, from which the data for this thesis was derived, received ethical approvals from multiple review boards. The study protocol was approved by the Comité de Recherche Clinique (CoRC) and the Institutional Review Board at the Institut Pasteur in Paris, France on October 25, 2018 and November 8, 2018, respectively. In Burkina Faso, the study was approved by the Comité d'éthique pour la recherche en santé (CERS) of the Ministère de la santé/Ministère de l'enseignement supérieur, de la recherche scientifique et de

l'innovation on December 4, 2018, the Comité d'éthique institutionnel du Centre MURAZ on April 4, 2019, and the Comité technique d'examen des demandes d'autorisation d'essais cliniques (CTEC) on November 4, 2019.

Informed consent was obtained from all participants involved in the original NéoVac and TADES studies. The informed consent process ensured that participants were provided with thorough information about the research in plain language, enabling them to make voluntary decisions about their participation.

By utilizing de-identified data from these previously approved studies, this thesis adhered to ethical principles of respect for persons, beneficence, and justice. The secondary analyses aimed to maximize the value of existing data while minimizing risks and burdens to participants.

Results

Temperature exposure at CSPS level. The continuously monitored temperature during the transportation of the vaccines from France to Burkina Faso was well maintained. Only one brief deviation occurred on June 30, 2020, between 08:30:51 and 18:40:51.

The data shown in (Table 1) summarizes the temperature exposures of 23 CSPS, excluding Moussobadougou due to missing temperature monitoring data from the Fridge-tag. The CSPS days were determined by adding up all of the days for every CSPS, starting from the day the CSPS received their first shipment of study vaccines. CSPS days with temperature data are all the days for each CSPS with Fridge-tag data available.

Fridge-tag data provided continuous daily minimum and maximum temperatures. The data also included the duration (hours) of when the temperature deviated from the recommended temperature range (2-8°C) for HepB-BD. The minimum and maximum temperatures were categorized into four mutually exclusive ranges: the recommended range of 2-8°C, temperatures only above 8°C, temperatures only below 2°C, and when both the maximum and minimum were below 2°C and above 8°C. The duration of temperature excursion from the recommended range was calculated for each CSPS.

The temperature data was available for 7,136 days (96.6%) out of the 7,390 total CSPS days. Overall, observed temperatures were within the recommended 2-8°C for 4,749 days (66.5%), while it was outside of this range for 2,386 days (33.5%). The minimum and/or maximum temperatures were above 8°C for 2,175 days (30.5%), possibly exposing vaccines to heat stress. In seventy-six (3.5%) of these occurrences, both the minimum and maximum temperatures were above 8°C. On the other hand, the minimum and/or maximum

temperatures were below 2°C for a total of 134 days (1.9%), while 78 days (1.1%) experienced both low and high temperature excursions. In one instance, in Yégueresso, both the minimum and maximum were below 2°C. The total number of hours the temperatures remained outside the 2-8°C range was 7,817 hours.

At the individual CSPS level, Koumi and Logofourousso had the highest percentage of days (88.9%) with the recommended temperature range, while Nasso had the highest proportion of days (65.4%) with temperatures above 8°C. Yégueresso experienced the most days (12.4%) with temperatures both below 2°C and above 8°C, indicating significant temperature instability. The longest duration (hours) of temperatures outside of the 2-8°C range occurred in Bouende (2,871.8 hours).

These findings suggest that while most CSPS's were able to maintain appropriate temperatures for the majority of the time, there were substantial periods where temperatures deviated from the recommended range. Some CSPS such as Yégueresso, Bouenda, Nasso, and Tiara, experienced fluctuating temperatures, promoting the study to replace the refrigerators of these locations.

Table 1: Distribution of Temperature Categories Across CSPS During the Observation Period

CSPS name	CSPS days N=7390	CSPS days with temp data N=7136	Recommended range (2-8°C) n=4749 (66.5%)	Only above 8°C n=2175 (30.5%)[2]	Only below 2°C n=134 (1.9%)[1]	Both below 2°C & above 8°C n=78 (1.1%)	Total hours of excursion (7817)
	n	n	n (%)	n (%)	n (%)	n (%)	hours
Sogossagasso	589	588	372 (63.3)	139 (23.6)	62 (10.5)	15 (2.6)	327
Koumi	568	557	495 (88.9)	62 (11.1)	0 (0.0)	0 (0.0)	33
Bouende	545	528	195 (36.9)	315 (59.7)	5 (0.9)	13 (2.5)	2871
Logofourousso	512	477	424 (88.9)	53 (11.1)	0 (0.0)	0 (0.0)	37
Baré	509	497	404 (81.3)	80 (16.1)	11 (2.2)	2 (0.4)	72
Kiri	483	443	332 (75)	110 (24.8)	1 (0.2)	0 (0.0)	89
Panamasso	452	428	305 (71.3)	122 (28.5)	0 (0.0)	1 (0.2)	109
Kotédougou	381	381	296 (77.7)	85 (22.3)	0 (0.0)	0 (0.0)	36
Karangasso/Sambla	421	420	249 (59.3)	167 (39.8)	1 (0.2)	3 (0.7)	516
Yégueresso	400	363	198 (54.5)	120 (33.1)	32 (8.8)	13 (3.6)	768
Gnafongon	372	372	186 (50.0)	186 (50.0)	0 (0)	0 (0.0)	367
Nasso	350	350	88 (25.1)	229 (65.4)	11 (3.2)	22 (6.3)	1754
Dodougou	335	309	175 (56.6)	134 (43.4)	0 (0.0)	0 (0.0)	255
Ouolokoto	175	156	123 (78.8)	32 (20.5)	1 (0.7)	0 (0.0)	18
Tiara	175	175	139 (79.4)	36 (20.6)	0 (0.0)	0 (0.0)	1
Léguéma	168	168	135 (80.4)	33 (19.6)	0 (0.0)	0 (0.0)	15
Santidougou	166	166	131 (79.0)	35 (21.0)	0 (0.0)	0 (0.0)	23
Peni	160	153	98 (64.0)	55 (36.0)	0 (0.0)	0 (0.0)	105
Kouentou	160	150	137 (91.3)	12 (8.0)	1 (0.7)	0 (0.0)	8
Kouakouale	132	129	69 (53.5)	60 (46.5)	0 (0.0)	0 (0.0)	107
Tapoko	124	116	66 (56.8)	32 (27.6)	9 (7.8)	9 (7.8)	82
Toussiana	95	92	48 (52.2)	44 (47.8)	0 (0.0)	0 (0.0)	205
Matourkou	118	118	84 (71.2)	34 (28.8)	0 (0.0)	0 (0.0)	21

[1] *One instance occurred where the daily minimum and maximum temperatures were both below 2°C during the observation period*

[2] *On 76 occasions, the daily minimum and maximum temperatures both exceeded 8°C throughout the observation period*

Child characteristics and vaccination data. As shown in (Table 2), the study sample involved 2,110 children, among whom 28 (1.3%) tested positive for HBsAg, a marker for hepatitis B infection. There were 1,040 (49.3%) girls and 1,069 (50.7%) boys received the hepatitis B birth dose. Most births, 2,034 (96.4%), occurred at health facilities, while 70 (3.3%) children were born at home. There were 6 (0.3%) premature births and 2,103 (99.6%) were born at a normal gestational age. The majority of the children had a normal birth weight

(≥2500g), while 306 (14.5%) were classified as low birth weight (<2500g). No significant differences were observed in the proportion of HBsAg-positive children based on sex (p=0.850), place of birth (p>0.900), birth weight (p=0.788) or gestational age (p>0.900).

There was a high rate of adherence to the full schedule of hepatitis B vaccinations, which includes HepB-BD and all three pentavalent HepB vaccines; with 1,979 (93.7%) children receiving all recommended doses. The timing of the birth dose, varied among the children: 627 (29.7%) received it within 12 hours after birth, 584 (27.7%) within 12-24 hours, 704 (33.4%) within 24-48 hours, 34 (1.6%) within 48-72 hours, and 161 (7.6%) after 72 hours. There were no difference in the proportion of children who are HBsAg-positive and the time they received the birth dose (p=0.371).

Children's HBsAg positivity was strongly associated with maternal hepatitis B status, assessed through maternal HBsAg testing, (p<0.0001). None of the children born to HBsAg-negative mothers tested positive for HBsAg compared to 27 (16.2%) of children born to HBsAg-positive mothers.

Table 2: Characteristics of Children and Hepatitis B Infection Status

Characteristis	Full Sample N= 2110	HBsAg Positive (n= 28)	HBsAg Negative (n= 2082)	p-value [1]
	n (%)	n (%)	n (%)	
Infant sex				
Female	1040 (49.3)	13 (1.3)	1027 (98.7)	0.850
Male	1069 (50.7)	15 (1.4)	1054 (98.6)	
Unknown [2]	1			
Place of birth				
Home	70 (3.3)	0(0)	70 (100)	>0.900
Health Facility	2034 (96.4)	28 (1.4)	2006 (98.6)	
Other	6			
Birth weight				
Normal birthweight (≥2500 g)	1803 (85.5)	25 (1.4)	1778 (98.6)	0.788
Low birthweight (<2500 g)	306 (14.5)	3 (1)	303 (99)	
Unknown [2]	1 (0.04)	0 (0.0)	1 (100)	
Gestational age				
Term	2103 (99.6)	28 (1.3)	2075 (98.7)	>0.900
Preterm	6 (0.3)	0 (0.0)	6 (100)	
Unknown [2]	1			
Heptatis B vaccination completion				
Received all 4 doses	1979 (93.7)	28 (1.4)	1951 (98.6)	0.368
Incomplete	103 (4.8)	0 (0.0)	103 (100)	
Unknown [2]	28			
Time to HepB birth dose				
≤12	627 (29.7)	12 (1.9)	615 (98.1)	0.371
12 to >24	584 (27.7)	6 (1.0)	578 (99.0)	
24 to >48	704 (33.4)	7 (1.0)	697 (99.0)	
48 to ≤72	34 (1.6)	1 (2.9)	33 (97.1)	
Greater 72	161	2 (1.2)	159 (98.8)	
Maternal HBsAg Status				
Positive	167 (7.9)	27(16.2)	140 (83.2)	<0.0001
Negative	1941 (92.1)	0 (0.0)	1941 (100)	
Unknown [2]	2			

[1] *Fisher's exact test*
[2] *Missing data for these variables*

HepB-BD vaccine temperature exposure and child HBsAg status. The relationship between HepB-BD vaccine temperature exposure and child HBsAg positivity was first examined using two different exposure classification schemes- stringent and flexible criteria (Table 3). Under the stringent exposure conditions, 110 (5%) were unexposed, always maintained within the recommended 2-8°C. 1 out of these 110 children (0.8%) tested HBsAg positive. 1559 children (74%) were exposed to temperatures greater than 8°C of which 22

(1.4%) in this group tested HBsAg positive. 441 (21%) children experienced mixed exposures, with temperatures both <2°C and >8°C. Among these children, 5 (1.1%) tested HBsAg positive. Children who received vaccines exposed to temperatures above 8°C and those with mixed temperature exposures were not statistically significant, (OR: 1.5, 95% CI: 0.1-5.5), (OR: 1.2, 95% CI: 0.2-24.1), respectively, when compared to children who were unexposed (always within 2-8°C).

The flexible exposure criteria yielded similar results. No children were exposed to low-risk temperatures of ≥ -0.5°C to 2°C or >8°C and >30°C. 103 (4.8%) children had vaccines that experienced high freezing risk temperatures greater than -0.5°C and 2 (2%) in this group tested HBsAg-positive. Children exposed to high heat risk temperatures >30°C were 34 (1.6%). None of these children tested HBsAg positive. The majority, 1,863 children (89.0%) were exposed to temperatures both below 2°C and above 8°C. In this group, 25 (1.1%) tested HBsAg positive. There were no statistically significant association between temperature exposure and HBsAg positivity when using the flexible exposure criteria, although 95% CIs were wide.

The median Cumulative Deviation Index (CDI), a measure of cumulative temperature exposure, was 7.6 (IQR:54.1) across all children. The median CDI was similar between HBsAg positive (10.3, IQR:23.6) and HBsAg negative (7.6, IQR:12.6) children. This difference was not statistically significant as determined by the Mann-Whitney U test (p=0.713).

Table 3: Hepatitis B Birth Dose Vaccine Exposure to Temperature Deviations and Children Hepatitis B Infection Risk

Characteristis	Full Sample Size N=2110	HBsAg Positive (n=28)	HBsAg Negative (n=2082)	OR (95% CI) [1]	p-value[1]
Exposure Conditions (°C)	**n(%)**	**n(%)**	**n(%)**		
Stringent exposure conditions					
Always within 2-8°C	110 (5.0)	1 (0.9)	109 (99.1)	ref	ref
Below 2°C but not exceeding 8°C	0 (0.0)	0 (0.0)	0 (0.0)	N/A[2]	N/A[2]
Above 8°C	1559 (74.0)	22 (1.4)	1537 (98.6)	1.5 (0.3, 28.1)	0.665
Mixed (below 2°C and above 8°C)	441 (21.0)	5 (1.1)	436 (98.9)	1.2 (0.2, 24.1)	0.839
Flexible exposure conditions					
Always within 2-8°C	110 (5)	1 (0.9)	109 (99.1)		ref
Expsure to low risk temperature					
Between ≥ -0.5°C and 2°C	0 (0.0)	0 (0.0)	0 (0.0)	N/A[2]	N/A[2]
Between 8°C and ≤ 30°C	0 (0.0)	0 (0.0)	0 (0.0)	N/A[2]	N/A[2]
Expsure to high risk temperature					
Freezing risk < -0.5°C	103 (4.8)	2 (2.0)	101 (98)	1.8 (0.2, 19.8)	0.567
Heat risk > 30°C	34 (1.6)	0 (0.0)	34 (100)	1.1(0.01, 20.3)	0.973
Exposed to both below 2°C and above 8°C	1863 (89.0)	25 (1.3)	1838 (98.7)	1.0 (0.3, 9.1)	0.988
Cumulative Deviation Index (CDI) [4]				**OR (95% CI) [3]**	**p-value[3]**
CDI (Median, IQR)	(7.6, 54.1)	(10.3, 23.6)	(7.6, 12.6)	1 (0.9, 1.0)	0.713
Log transformed CDI (Median, IQR)	(1.3, 4.8)	(2.3, 2.2)	(2, 3.4)	1 (0.9, 1.1)	0.714

[1] Firth's penalized likelihood model; OR, odds ratio; CI, confidence interval; p-value

[2] NA, not applicable due to zero counts in these categories

[3] *Logistic regression model; OR, odds ratio; CI, confidence interval; p-value*

[4] *Cumulative Deviation Index (CDI): Novel metric integrating both the duration and severity of temperature deviations, with weighting factors based on vaccine stability studies (0 for 2-8°C, 1 for >8 to <20°C, 2 for >20 to <37°C, 3 for >37°C, 4 for <2 to <-0.5 °C, and 6 for <-0.5°C), calculated by multiplying the exposure duration (hours) in each category by the corresponding factors and summing these values for each vaccine*

Maternal HBsAg status and child HBsAg positivity. The analysis presented in (Table 4) focused specifically on 167 children born to HBsAg-positive mothers, 27 (16.2%) of whom tested positive for HBsAg. Vaccine temperature exposures during storage were examined, with the majority 127 (76.0%) receiving HepB-BD stored within the 8.1-20.0°C range. Smaller proportions were exposed to temperatures equal to/below 0°C or temperatures above 20°C, 17 (10.2%) and 12 (7.2%), respectively. There was no statistically significant difference between the proportion of children who tested positive for HBsAg in the various temperature categories (p=0.816).

High maternal HBV DNA viral load, defined as ≥5.3 IU/mL (≥200,000 IU/mL), was a significant predictor of child hepatitis B infection. Children born to mothers with a high viral load were 7.8 (95% CI: 2.5-24.4, p-value: 0.0003) times more likely of test HBsAg positive compared to children born to mothers with lower maternal loads. Consequently, the analysis of temperature exposures during vaccine storage was adjusted for maternal viral load to account for this critical risk factor.

The stringent temperature exposure conditions showed that the majority, 129 (77.2%), of the vaccines were exposed to temperatures above 8°C, and 27 (16.2%) of the vaccines were exposed to mixed temperatures (2°C and above 8°C). After adjusting for maternal HBV viral load, the odds ratios were 1.8 (95% CI: 0.3-19.9, p-value: 0.549) and 1.1 (95% CI: 0.1-15.4, p-value: 0.941) for temperatures above 8°C and for mixed temperatures (2°C and above 8°C), respectively. There were no statistically significant associations between temperature exposure and HBsAg positivity when using the stringent exposure criteria, although 95% Cis were wide, indicating a high degree of uncertainy.

Temperatures were categorized below or equal to 0°C and greater than 0°C. After adjusting for maternal viral load, the odds of children testing positive for HBsAg was 1.1 higher in children who received a vaccine exposed to temperatures below or equal to 0°C. However, this was not statistically significant (95% CI: 0.1-5.5, p-value: 0.902).

To capture the cumulative temperature excursion, a novel Cumulative Deviation Index (CDI) metric was calculated by integrating the duration and degree of temperature deviations from the recommended range during vaccine storage. HBsAg-positive children had a higher median log CDI (median: 2.5, IQR: 2.3) compared to HBsAg-negative children (median: 1.7, IQR: 3.2). The odds ratio of 1.0 (95% CI: 0.9-1.2, p-value: 0.714) with a p-value greater than threshold p-value (0.05) suggests there is no significant association between the log CDI vaccines experienced and HBsAg positivity in children, after adjusting for maternal viral load.

High maternal hepatitis B viral load emerged as the primary risk factor for child HBsAg positivity, while no significant association was found between vaccine exposures during storage and child HBsAg status.

Table 4: Effectiveness of HepB-BD Vaccine Stored OCC among Children Born to HBsAg-Positive Mothers

Characteristis	Full sample size N= 167	HBsAg Positive n= 27 [1]	HBsAg Negative n= 140	p-value [2]			
Temperature exposure	**n (%)**	**n (%)**	**n (%)**				
≤ 0°C	17 (10.2)	4 (23.5)	13 (76.5)	0.816			
0.1 to 8.0°C	11 (6.6)	1 (9.0)	10 (91.0)				
8.1 to 20.0°C	127 (76.0)	20 (15.7)	107 (84.3)				
≥ 20.1 °C	12 (7.2)	2 (16.7)	10 (83.3)				
Stringent exposure conditions				**Unadjusted model [2]**		**Adjusted model [2]**	
				OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Always within 2-8°C	11 (6.6)	1 (9.1)	10 (90.9)	ref		ref	
Below 2°C but not exceeding 8°C	0	0	0	N/A[7]	N/A[7]	N/A[7]	N/A[7]
Above 8°C	129 (77.2)	22 (17.1)	107 (82.9)	1.5 (0.3, 14.1)	0.659	1.8 (0.3, 19.9)	0.549
Mixed (below 2°C and above 8°C)	27 (16.2)	4 (14.8)	23 (85.2)	1.3 (0.2, 14.6)	0.768	1.1 (0.1, 15.4)	0.941
				Unadjusted model [3]		**Adjusted model [3]**	
Temperature exposure				**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
> 0°C	150 (89.8)	23 (15.3)	127 (84.7)	ref		ref	
≤ 0°C	17 (10.2)	4 (23.5)	13 (76.5)	1.7 (0.4, 5.3)	0.389	1.1 (0.1, 5.5)	0.902
Maternal viral load [4]							
<5.3 (IU/ml)	117 (70.1)	12 (10.3)	105 (89.7)	ref			
≥5.3 (IU/ml)	17 (10.2)	8 (47.1)	9 (52.9)	7.8 (2.5, 24.4)	0.0003		
Unknown [3]	33	7	36				
Cumulative Deviation Index (CDI) [5]	**Median (IQR)**						
Log transformed CDI	1.8 (3.1)	2.5 (2.3)	1.7 (3.2)	1.0 (0.9, 1.2)	0.324	1.0 (0.9, 1.2)	0.512

[1] One child who was positive for HBsAg has mother with unknown HBsAg status

[2] Firth's penalized likelihood model; OR, odds ratio; CI, confidence interval; p-value

[3] Logistic regression model; OR, odds ratio; CI, confidence interval; p-value

[4] The 200,000 IU/mL (5.3 log10) HBV viral DNA load cutoff indicates increased mother-to-child transmission risk and disease progression, warranting treatment initiation per WHO guidelines; 33 mothers had unknown HBV viral DNA load

[5] Cumulative Deviation Index (CDI): Novel metric integrating both the duration and severity of temperature deviations, with weighting factors based on vaccine stability studies (0 for 2-8°C, 1 for >8 to <20°C, 2 for >20 to <37°C, 3 for >37°C, 4 for <2 to <-0.5 °C, and 6 for <-0.5°C), calculated by multiplying the exposure duration (hours) in each category by the corresponding factors and summing these values for each vaccine

[6] Adjusted model including mother HBV viral DNA load. OR, odds ratio; CI, confidence interval

[7] NA, not applicable due to zero counts in these categories

Anti-HBs antibody titer. The subset TADES study population included 68 children, 26 who had received the monovalent HepB-BD as part of the NéoVac study and 42 who did not receive HepB-BD. Serological outcomes were assessed by measuring anti-HBs titers, a marker of vaccine-induced immunity. Seroprotection was defined as anti-HBs levels ≥10 mIU/mL measured 4 weeks after the third dose of the pentavalent HepB vaccine.

As shown in (**Error! Reference source not found.**) among children who completed the full schedule of HepB vaccine (including HepB-BD), 20 (91.0%) had protective levels of anti-HBs titers ≥10 mIU/mL, compared to those who received HepB-BD but missing some doses of the HepB3, 2 (50.0%, p=0.098). For children receiving all the HepB3 doses, pentavalent DTP-HepB-Hib series, without birth dose, seroprotection rates were lower 14 (77.8%) for ≥10 mIU/mL and 6 (33.3%) for ≥ 100 mIU/mL.

Table 5: Comparing Anti-HBs Titer by Vaccination Status and Vaccine Storage Conditions

Characteristics	N=26	Anti-HBS titre ≥10 [1]	Anti-HBS titre <10 [1]	p-value [2]		Anti-HBS titre ≥100 [1]	Anti-HBS titre <100 [1]	p-value [2]	
In those received HepB-BD, completed all HepB3 [4]	**n (%)**	**n (%)**	**n (%)**			**n (%)**	**n (%)**		
Yes	22 (84.6)	20 (91.0)	2 (9.0)	0.098		17 (77.0)	5 (23.0)	0.287	
No	4 (15.4)	2 (50.0)	2 (50.0)			2 (50.0)	2 (50.0)		
In those without HepB-BD, completed all HepB3 [4]	**N=42**								
Yes	18 (42.9)	14 (77.8)	4 (22.2)	0.505		6 (33.3)	12 (66.7)	0.530	
No	24 (57.1)	16 (66.7)	8 (33.3)			11 (45.8)	13 (54.2)		
Restricted to those who completed all DTP-HepB-Hib vaccine									
Characteristics		**Anti-HBS titre ≥10 [1]**	**Anti-HBS titre <10 [1]**	**p-value [2]**		**Anti-HBS titre ≥100 [1]**	**Anti-HBS titre <100 [1]**	**p-value [2]**	
Stringent exposure conditions	**n= 22**								
Always within 2-8°C	2 (9.0)	2 (100)	0	0.315		1 (50.0)	1 (50.0)	0.822	
Below 2°C but not exceeding	0 (0.0)	0 (0.0)	0 (0.0)			0 (0.0)	0 (0.0)		
Above 8°C	20 (91.0)	18 (90.0)	2 (10.0)			16 (80.0)	4 (20.0)		
Mixed (below 2°C and above	0 (0.0)	0 (0.0)	0 (0.0)			0 (0.0)	0 (0.0)		
Vaccination Status	**n**			**OR (95% CI) [3]**	**p-value [3]**			**OR (95% CI) [3]**	**p-value [3]**
No HepB-BD [4]	18	14 (77.8)	4 (22.2)	ref		6 (33.3)	12 (66.7)	ref	
HepB-BD stored OCC [4]	20	18 (90)	2 (10.1)	2.3 (0.4, 14.8)	0.326	16 (80.0)	4 (20.0)	7.1 (1.8, 31.8)	0.004
HepB-BD stored ICC [4]	2	2 (100)	0 (0.0)			1 (50.0)	1 (50.0)		

[1] *Anti-HBs (Hepatitis B surface antibody)*
[2] *Fisher's exact test*
[3] *Firth's penalized likelihood; p-value; OR, odds ratio; CI, confidence interval*
[4] *OCC, out-of-cold chain; ICC, inside-cold chain; HepB-BD, hepatitis B birth dose vaccine; DTP-HepB-Hib, pentavalent hepatitis B vaccine*

The exploratory analyses, visualized through a scatterplot (Figure 3), shows a weak negative correlation (r= -0.37) between the cumulative deviation index (CDI), which represents a prolonged exposure to out-of-cold-chain temperatures, and the logarithm base 10 of anti-HBs titer. This suggests a slight decrease in antibody response with increased CDI. The association between the lowest temperature each vaccine was exposed to and the logarithm of anti-HBs titer, showed a weak negative correlation (r= -0.12) (Figure 4). In contrast, the association between the highest temperature vaccines experienced and the logarithm of anti-HBS titre, shows a weak positive linear association (r= 0.14), (Figure 5).

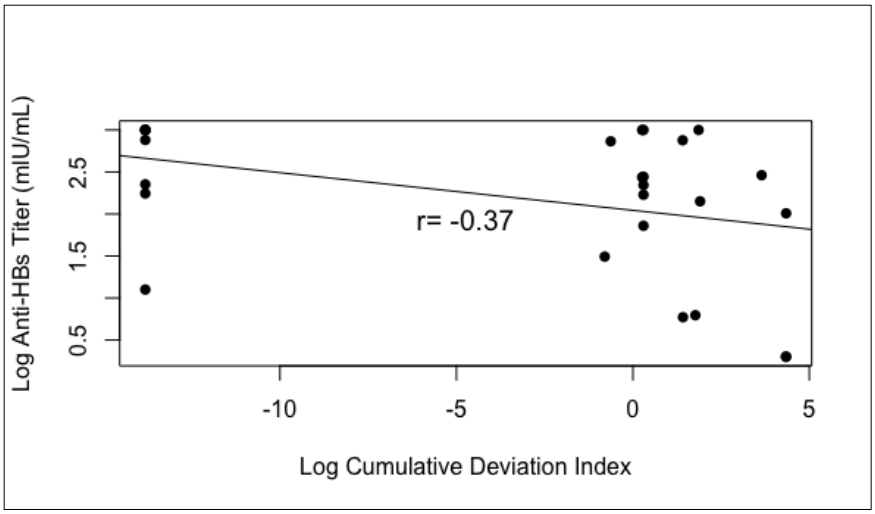

Figure 3: Relationship between Log Cumulative Deviation Index and Log Anti-HBs Titers

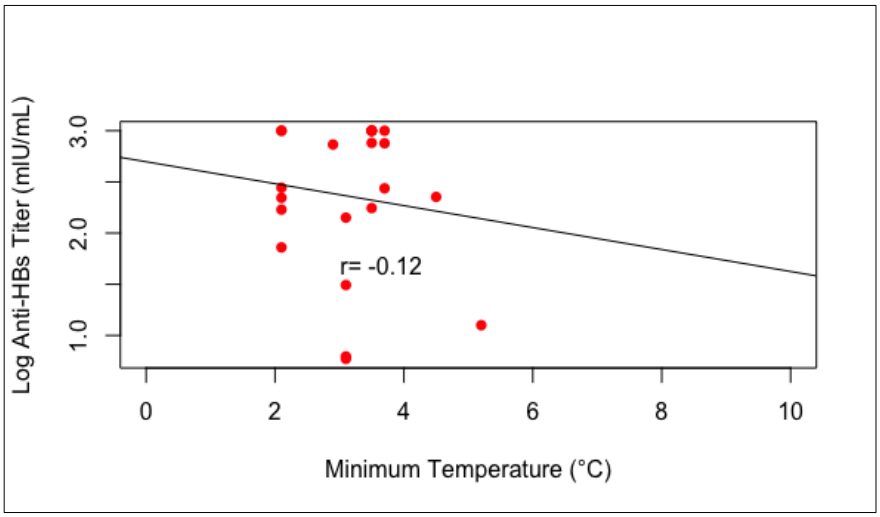

Figure 4: Relationship Between Minimum Temperature and Anti-HBs Titers

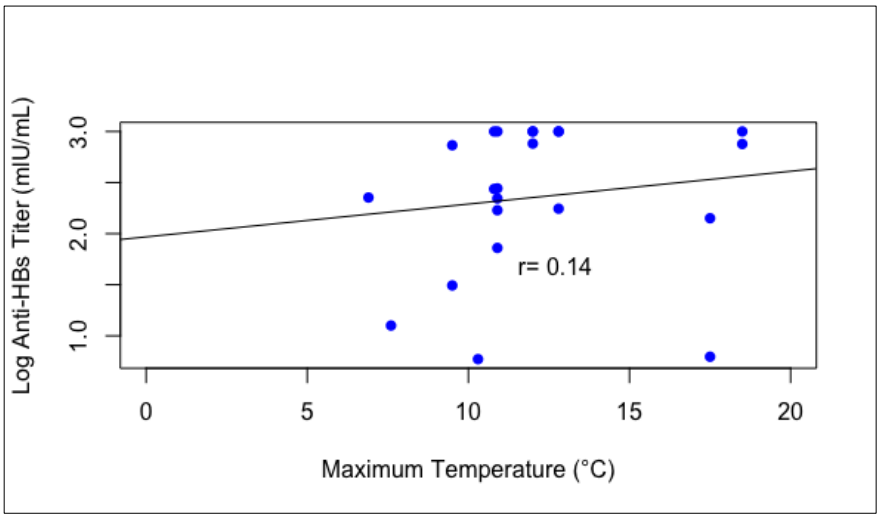

Figure 5: Relationship Between Maximum Temperature and Anti-HBs Titers

To assess the immunogenicity of the hepatitis B vaccine in children who received the HepB-BD vaccine exposed to temperatures outside the recommended cold chain parameters, the levels of anti-HBs of children with OCC birth dose were compared to children who did not receive the birth dose. For better comparability, analyses in both groups were restricted to children who received all the other three pentavalent doses HepB3 vaccine. We employed a boxplot (Figure 6) to compare the logarithm of anti-HBs titer (mIU/mL) across three groups; children who received the birth dose of the vaccine under recommended temperature (n=2), children who received the birth dose but were exposed to OCC temperatures, and children who did not receive the birth dose at all. The OCC group had the highest median of anti-HBs titer compared to the group without the birth dose. It's important to acknowledge that the group within the recommended temperature had a very small sample size.

The majority, 91.0% (20), experienced temperatures above 8°C while 9.0%(2) had temperatures always within the recommended range (2-8°C). No vaccines experienced temperatures below 2°C but not exceeding 8°C and mixed exposure conditions (below 2°C and above 8°C). The p-value indicates no significant differences between the exposure groups for both anti-HBs titer thresholds of ≥10 mIU/mL (p=0.315) and ≥100mIU/mL (p=0.822).

Despite these temperature excursions, children who received the HepB-BD, even when stored OCC, demonstrated higher odds of having anti-HBs antibody titer ≥ 100 mIU/mL compared to those who missed the birth dose. Those who had HepB-BD stored OCC had a 7.1 times greater likelihood of having seroprotection ≥ 100 mIU/mL (95% CI 1.8-31.8, p=0.004) compared to those children who did not receive the HepB-BD vaccine (Table 5).

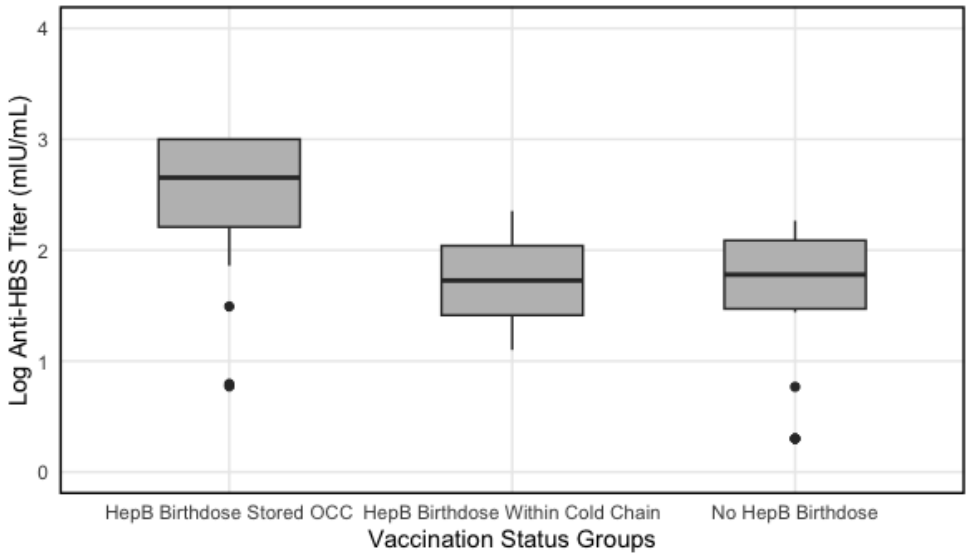

Figure 6: Comparing the Log Anti-HBS Titer by the Vaccination Status of Children who Completed HepB3

Discussion

To our knowledge, this is the first study assessing the real-world temperature exposures of hepatitis B birth dose (HepB-BD) vaccines and the impact of temperature deviations on vaccine effectiveness and immunogenicity in Africa. By leveraging robust continuous temperature monitoring data from Fridge-tag devices and directly linking it to serological outcomes, hepatitis B surface antigen (HbsAg) positivity, and anti-HBs antibody titer, we provide insights into the relationship between vaccine effectiveness, ***immunogenicity,*** cold chain adherence, and immune responses.

The temperature monitoring data from the 23 CSPS showed major deviations from the recommended cold chain conditions. Although the maximum and minimum temperatures were

within the recommended range for 66.5% of the days, temperature excursions occurred: 30.5% of the days had temperatures above 8°C, 1.9% below 2°C, and 1.1% with both maximum and minimum exceeding 8°C and falling below 2°C. These findings align with previous studies illustrating cold chain problems in low and middle-income countries (29, 30).

Maternal HBsAg serostatus and viral DNA load were the primary risk factors for child hepatitis B infection, with higher viral loads leading to a 7.8 times higher likelihood of child infection. Because of this, analyses were stratified to HBsAg-positive mothers. After adjusting for this risk factor, no significant association between vaccine temperature excursion exposures and child HBsAg status was found. One novel aspect of the study was the calculation of the Cumulative Deviation Index (CDI), a metric that aims to capture the cumulative temperature excursions experienced by the vaccine during storage. HBsAg-positive children had a higher median CDI compared to HBsAg-negative children, but after adjusting for the critical risk factor of maternal viral load, there was no significant association between the CDI and child HBsAg positivity.

Despite the temperature deviations, the serological data showed that children receiving the OCC Hep-BD vaccine had higher proportions of seroprotection, defined as anti-HBs titer $\geq$10 mIU/mL(90.0%), compared to those who did not receive birth dose (77.8%). The difference was even more pronounced when comparing the anti-HBs titer $\geq$100 mIU/mL. Children with the OCC Hep-BD vaccine had much higher anti-HBs proportions of $\geq$100 mIU/mL (80.0%) compared to those who did not receive the birth dose (33.3%). Notably, even when the HepB-BD vaccines were exposed to temperature deviations above 8°C, Children had a 7.1-fold higher likelihood of achieving anti-HBs titers $\geq$100 mIU/mL compared to those who missed the birth dose. It's important to note that in this group, no vaccine was exposed to below 2°C.

The results of this study are congruent with previous studies and provide further evidence of the immunogenicity of HepB vaccine after exposure to temperature deviations. Several studies, including the WHO systematic review, have demonstrated the thermostability of the HepB vaccine, which elicits a protective level of anti-HBs even after exposure to temperatures up to 37°C. Field studies are consistent with our findings, showing that immunogenicity of the vaccine remained after the birth dose was stored at ambient or elevated temperatures, compared to those strictly maintained within the cold chain.

Implications

The findings from this study have significant implications for improving access to the HepB birth dose in low-resource settings. The data demonstrated that the HepB monovalent vaccine

maintained its effectiveness even when exposed to temperature deviations outside the recommended 2-8°C range. It was observed that children receiving the OCC Hep-BD had higher proportions of seroprotection compared to those who missed the birth dose, even when vaccines were exposed to temperatures above 8°C. This includes a 7.1-fold higher likelihood of having anti-HBs titers ≥100 mIU/ML among those vaccinated, despite the temperature excursions. Previous studies showed that an OCC policy led to an increase in HBV birth dose coverage, with some studies reporting as high as a 27% increase (22-24).

Conclusion

In summary, this study provides evidence from a real-life field context that the hepatitis B birth dose vaccine remained immunogenic despite the temperature excursion above the recommended ranges, likely due to its inherent thermostability. Implementing controlled temperature chain strategies levering the vaccine's heat stability could improve birth dose accessibility in areas with cold chain constraints, contributing to the prevention of mother-child transmission

Limitations

- The primary assumption of this study when linking the vaccines to the children was that vaccines were assigned on a First-In-First-Out basis. If this assumption was violated, it could lead to misclassification of the vaccine exposures to temperatures. The next step of this study is to conduct a sensitivity analysis that would assign new vaccines first, on a Last-In-First-Out basis, to address any potential violations of this assumption.
- Some temperature monitoring data from the CSPS were lost, making it challenging to link all vaccines.
- There were two readings of temperature recorded daily, minimum and maximum. However, it was not possible to know how long the temperature remained in the lowest or highest daily temperatures as the duration (hours) of the excursion was counted when the temperature deviated from 2-8°C

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